

Short Communication

The Growth of and Organ Formation from Callus Tissue of *Sorghum*¹

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Successful culturing of callus has recently been reported for the following monocotyledonous species: corn (Reference 2, and Linsmaier Bednar, E. M., personal communication), oats (1, 6), rice root (8), asparagus (7), wheat (5), and sorghum (4).

For the culture of sorghum callus from roots and tillering nodes, Strogonov *et al.*, (4) used Murashige and Skoog's basal medium plus calcium pantothenate, 5 mg/liter; 2,4-dichlorophenoxyacetic acid, 1 mg/liter; kinetin, 1 mg/liter; casein hydrolysate, 0.5 g/liter; and ascorbic acid, 1 mg/liter. The same basal inorganic medium was also used for the culture of oats (1), asparagus (7), and by Linsmaier Bednar for corn. Corn callus was also cultured on White's medium (2), oats and rice root on Heller's medium (6, 8), and wheat on Hildebrandt's D medium (5). Generally, 2,4-D was used as the auxin, but α -naphthaleneacetic acid as well as 2,4-D was used for corn (2). In other respects the same nutrients and growth conditions were used as those required for the culture of dicotyledonous callus.

The ability of monocotyledonous callus tissue to form organs has been demonstrated by the production of embryoids and plantlets from asparagus cells (7), many shoots but few roots from oats (1), roots from wheat callus (5), and roots (2) and both roots and shoots from corn callus (Linsmaier Bednar, E. M., personal communication).

This report describes the culture of sorghum callus, the initiation of shoot primordia, and development of plants from callus tissue of this species.

MATERIALS AND METHODS

Sorghum bicolor Moench, var. Norghum and North Dakota 104, were used in this study. The seeds were surface-sterilized with sodium hypochlorite (1 part Clorox to 9 parts water) for 10 to 30 min and were germinated for 5 to 7 days in the dark with distilled water in sterile Petri dishes. The shoot was excised just below the first node and placed on Murashige and Skoog's (3) 1962 revised basal medium with 2.5% (w/v) sucrose, coconut milk 10% (v/v, prepared from coconuts on the market), and 2,4-D at various concentrations. The medium was adjusted to pH 5.6 to 5.8 with NaOH or HCl before autoclaving.

Callus cultures were grown at 22 to 25 C in weak, diffuse light in the laboratory or in growth chambers with cool white fluorescent light (800 ft-c and 14-hr day length) for shoot initiation. Cultures were grown in 125-ml Erlenmeyer flasks with 40 ml of agar medium or in solubility tubes with 25 ml of liquid medium on a wheel rotating at 4 or 10 rpm.

RESULTS

Sorghum seeds germinated in 2,4-D solutions showed a marked decrease in length of shoots and roots in the concentration range between 0.01 and 0.1 mg/liter. Root length was affected most and was completely inhibited at 20 mg/liter 2,4-D. From these results an experiment was run using excised sorghum shoots on M and S (1962) medium with coconut milk and a concentration range of 0.1 to 20 mg/liter 2,4-D. The range of 1 to 5 mg/liter 2,4-D was found to be optimal for callus initiation on the first node of excised shoots (Fig. 1, A, B, C).

A compact, yellow callus tissue forms on the first node of sorghum shoots and can be subcultured after 2 to 3 weeks. The growth of the transferred callus was slow and variable, but after several months in culture callus of the type D or E (Fig. 1) was obtained. Sorghum callus cultured by this method characteristically produced a pigment which turned both the callus and medium black (Fig. 1D).

Indole-3-acetic acid or naphthaleneacetic acid at concentrations equal to or higher than 2,4-D failed to produce callus tissue. Callus could be grown on medium without coconut milk but at a much slower rate. Kinetin at 1 mg/liter substituted for coconut milk permitted some, but very slow, growth of the callus.

In the second and third passages of the callus on solid medium with low concentrations of IAA, 2,4-D, or α -naphthaleneacetic acid, root formation occurred as small white bumps on the surface of the cultures. In liquid medium with 0 to 1 mg/liter 2,4-D, roots also formed. Attempts to subculture the roots on either solid or liquid medium failed. In liquid medium many single cells and cell clusters were obtained, but no bud or embryo formation was observed. Vacuoles, active cytoplasmic streaming, and inclusion bodies (Fig. 2) were found in single cells, when viewed under phase contrast (light) microscopy. The inclusion bodies, designated brown bodies, were also numerous in blackened callus tissue.

Callus tissue initiated and subcultured twice on agar medium with 2,4-D and coconut milk consisted of some white and some blackened tissue. When transferred to a medium with 5 mg/l α -naphthaleneacetic acid substituted for 2,4-D and cultured in growth chambers with 800 ft-c light and a 14-hr day length, the white tissue soon turned green and within 1 week buds were observed (Fig. 1F). These buds formed a few roots. When transferred to pots with vermiculite, watered with Hoaglands solution, and grown under greenhouse conditions, the buds formed plantlets which were eventually grown to maturity (Fig. 1G).

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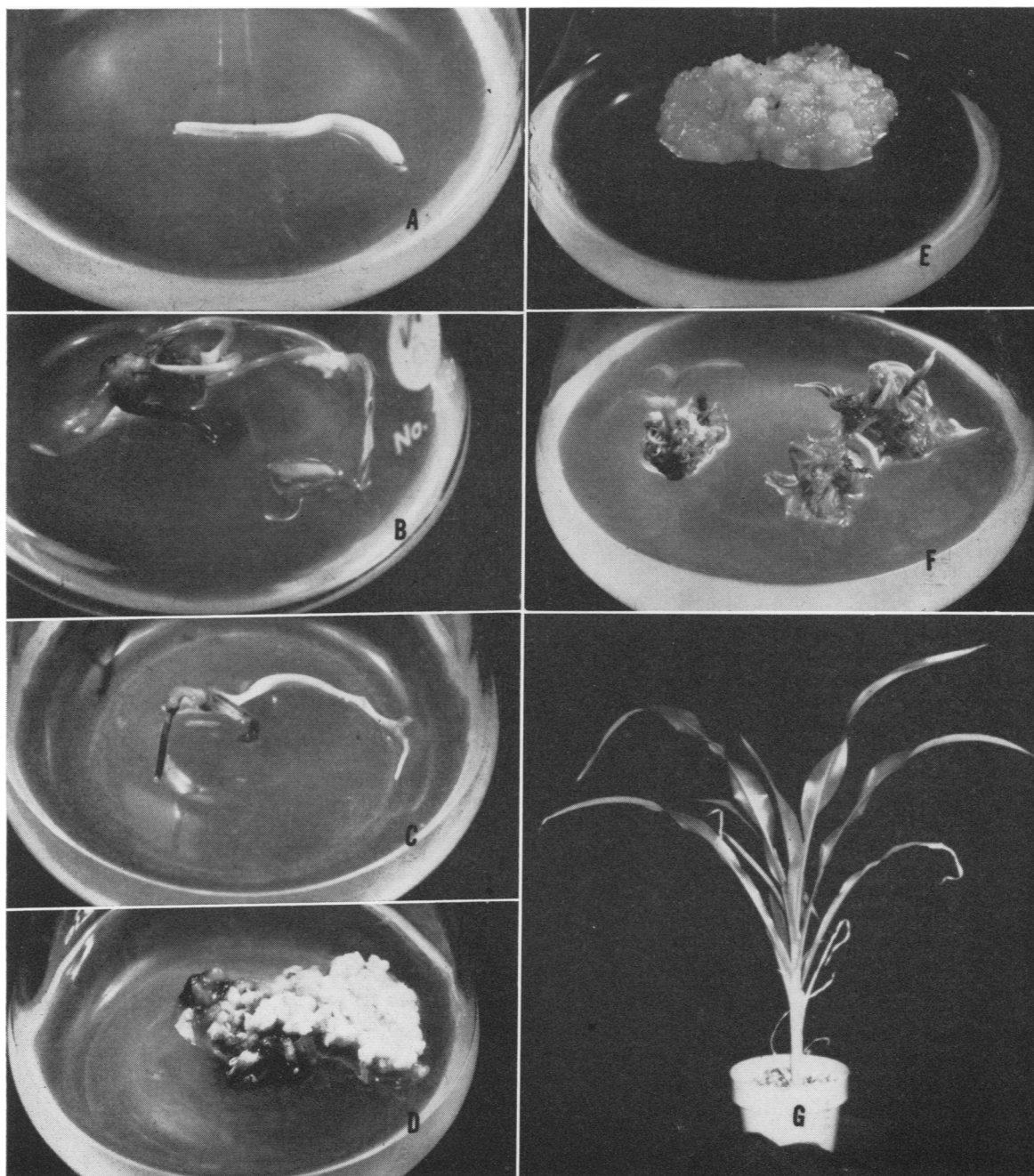


FIG. 1. Stages in the development of callus and organ formation in sorghum. A: Starting material (first node, 0 mg/liter 2,4-D) ($\times 1$); B: callus at the first node at 5 mg/liter 2,4-D ($\times 1$); C: callus at 15 mg/liter 2,4-D ($\times 1$); D: subculture of callus showing white, yellow, and black tissue at 5 mg/liter 2,4-D ($\times 1$); E: subculture showing homogenous yellow callus at 5 mg/liter 2,4-D; F: subculture from white callus placed in light and at 5 mg/liter α -naphthaleneacetic acid showing organ formation ($\times 1$); G: plantlets transferred to pot containing vermiculite and watered with Hoagland's solution.



FIG. 2. Single cells produced from sorghum callus when placed on shake culture. Note most cells have one to two large brown bodies. $\times 300$

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